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ENZYMATIC HYDROLYSIS OF CELLULOSIC WASTES TO GLUCOSE

By

L. A. Spano
J. Medeiros
M. Mandels

Pollution Abatement Division
Food Sciences Laboratory

8 September 1975

UNITED STATES ARMY
NATICK DEVELOPMENT CENTER
NATICK, MASSACHUSETTS 01760

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REPORT DOCUMENTATION PAGE

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
08-09-1975	Final	June 1972 – June 1975

4. TITLE AND SUBTITLE

ENZYMATIC HYDROLYSIS OF CELLULOSIC WASTES TO GLUCOSE

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

L.A. Spano, J. Medeiros, and M. Mandels

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Natick Soldier Research, Development and Engineering Center
ATTN: RDNS-WSC-C
Kansas St., Natick, MA 01760-5020

8. PERFORMING ORGANIZATION REPORT NUMBER

NATICK/SP-75/001

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This report summarizes a study performed by the U.S. Army Natick Development Center in the 1970s of the feasibility and advantages of using enzymes to hydrolyze cellulose waste to glucose as an alternative to using acid in this process, which was designed to reduce the amount of disposable solid waste by recycling it for other uses. The degree of enzymatic conversion of waste cellulosic materials depends upon both the source and the history of the waste cellulosics. For example, within 48 hours, conversion of pure cotton cellulose was only 10% whereas some of the waste cellulosic material such as pulped documents was as high as 80% conversion to glucose syrups. Suggestions as to the appropriate level of enzyme loading and the operational conditions needed for both the fermentation to produce the enzymes and the conditions required for adequate hydrolysis of the cellulosic materials are provided. The technology of producing the fungal enzymes and the subsequent enzymatic hydrolysis of cellulosic wastes was demonstrated at the pilot plant level.

15. SUBJECT TERMS

FUNGI	GLUCOSE	HYDROLYSIS	CULTURES(BIOLOGY)
ENERGY	CELLULOSE	FERMENTATION	TRICHODERMA VIRIDE
ENZYMES	FILTRATION	CELLULOSIC WASTES	ENZYMATIC HYDROLYSIS

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Alfred Allen
U	U	U	SAR	31	**19b. TELEPHONE NUMBER** *(include area code)* 508-233-4352

Standard Form 298 (Rev. 8-98)
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ENZYMATIC HYDROLYSIS OF CELLULOSIC WASTES TO GLUCOSE

By

L. A. Spano, J. Medeiros, M. Mandels
U. S. ARMY NATICK DEVELOPMENT CENTER
Natick, MA 01760

Cellulose is our most abundant organic material which can be used as a source of food, fuel and chemicals. The net world-wide production of cellulose is estimated at one hundred billion tons per year. This is approximately 150 lbs. of cellulose per day for each and every one of the earth's 3.9 billion people. The energy to produce this vast quantity of cellulose comes from the sun and is fixed by photosynthesis.

The energy from the sun, available over the United States alone is between 4 and 5 X 10^{19} BTU/Yr. This is approximately 600 times the annual energy consumption of the United States. Prior to 1900, our principal sources of energy were the wind, wood, water power and coal. During this century we have been relying very heavily on fossil fuels originally produced by photosynthesis. Our energy consumption in the United States has been estimated at 7 to 8 X 10^{16} BTU/Yr. This total energy is obtained primarily from oil (43%), gas (35%), and coal (19%). (1). Comparison of the annual energy consumption in 1873 (4.2 X 10^{15} BTU/Yr) with that of today, shows that our current demand is approximately seventeen to twenty times more than what we used in 1873. This phenomenal growth in energy demand will be difficult if not impossible to support with our current fuel reserves regardless of processing capabilities. By year 2000, undoubtedly nuclear power will be a major source of energy; however, to achieve the ultimate goal of independence, we will have to harness effectively and economically the inexhaustible energy of the sun.

Since cellulose is the only organic material that is annually replenishable in very large quantities, we must explore ways to utilize it as a source of energy, food, or chemicals. The utilization of this resource is greatly simplified if cellulose is first hydrolyzed to its monomer glucose as shown in Figure 1. Once we have the glucose, it can be used as a food consumable by man and animals, it can be converted to chemical materials, it can be converted microbially into single cell proteins, or it can be fermented to clean

CELLULOSE - A CHEMICAL AND ENERGY RESOURCE

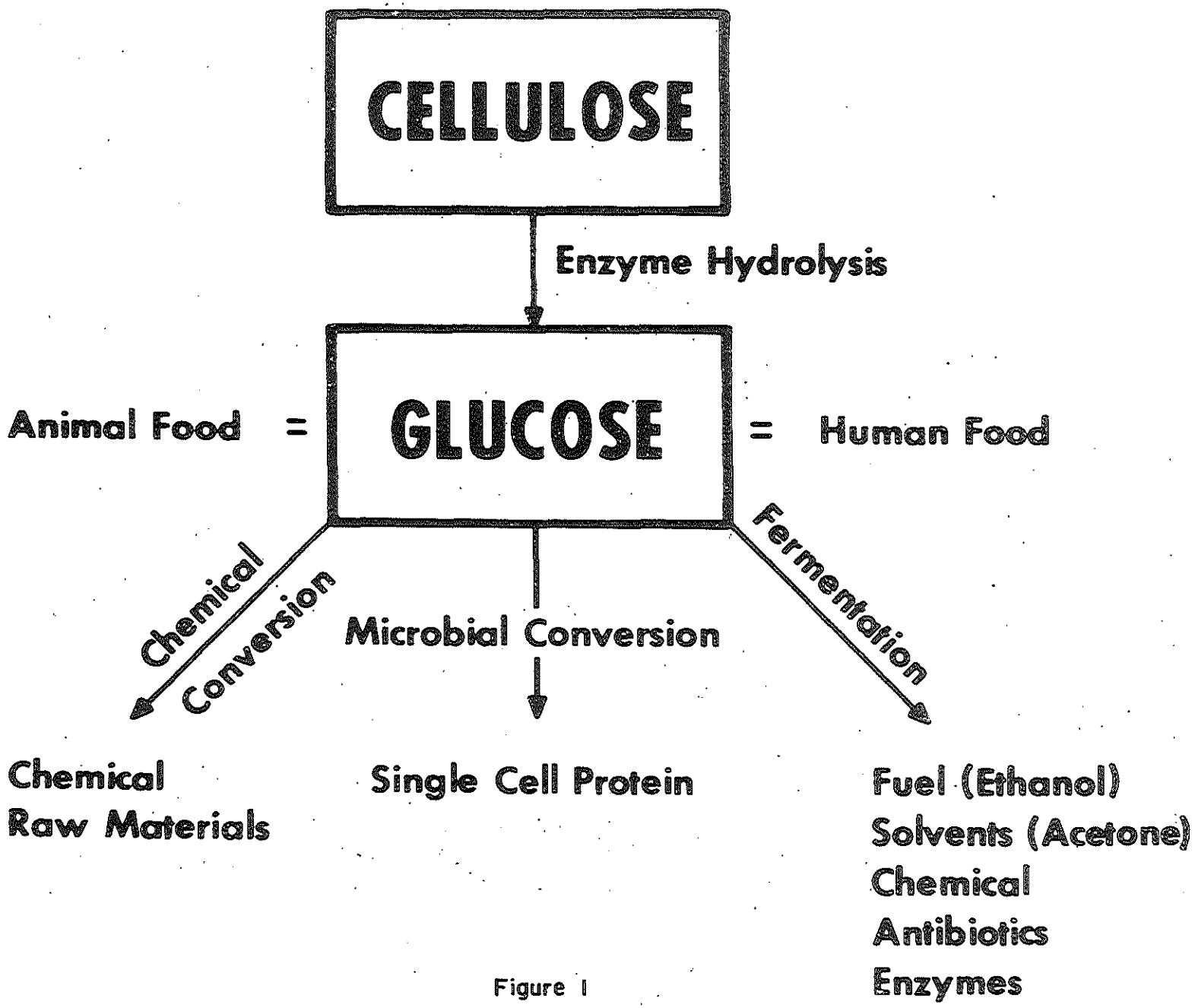

Figure 1

burning fuel (ethanol, solvents, acetone), and other chemicals. It is estimated that from one ton of waste paper, we can produce 1/2 ton of glucose which can be fermented to produce 68 gallons of ethanol.

Conversion of cellulose to glucose can be done by either acid hydrolysis or by enzymatic processes. (2-12). There are various advantages in the use of enzymes to hydrolyze cellulose instead of acid. When using acid, expensive corrosion-proof equipment is required. Moreover the crystalline structure of cellulose makes it very resistant to acid so that the temperature and acid concentration needed to achieve hydrolysis also cause decomposition of the resulting sugars. Consequently, the process must be balanced so that the rate of hydrolysis must be sufficiently high to compensate for the decomposition of the desired products. Glucose yields of approximately 50% of the weight of cellulose used have been obtained. (14). Waste cellulose invariably contains impurities which will react with the acid thereby producing other unwanted by-products and reversion compounds.

The enzyme on the other hand is specific for cellulose and does not react with impurities that may be present in the waste. Moreover reaction takes place at moderate conditions so that the glucose yield is 111% of the weight of cellulose used. The glucose syrups produced enzymatically are fairly pure and constant in composition.

We at the U. S. Army Natick Development Center, are developing an enzymatic process, which is based on the use of the cellulase derived from mutant strains of the fungus *Trichoderma viride* isolated and developed at the Natick Development Center. A schematic diagram of this process is shown in Figure 2. The first step is the production of the enzyme. This is accomplished by growing the fungus *Trichoderma viride* in a culture medium containing shredded cellulose and various nutrient salts. Following its growth, the fungus culture is filtered and the solids discarded. The clear straw colored filtrate is the enzyme solution that is used in the saccharification reactor. Prior to its introduction into the reactor, the enzyme broth is assayed for cellulase and its acidity adjusted to a pH of 4.8. Milled cellulose is then introduced into the enzyme solution and allowed to react with the cellulase to produce glucose sugar. You will note that saccharification takes place at atmospheric pressure and at a temperature of 50°C. The unreacted cellulose and enzyme is recycled back into the reactor, and the crude glucose syrup is filtered for use in chemical, or microbial fermentation processes to produce chemical feedstocks, single cell proteins, fuels, solvents, etc.

ENZYMATIC CONVERSION OF WASTE CELLULOSE TO GLUCOSE SUGAR

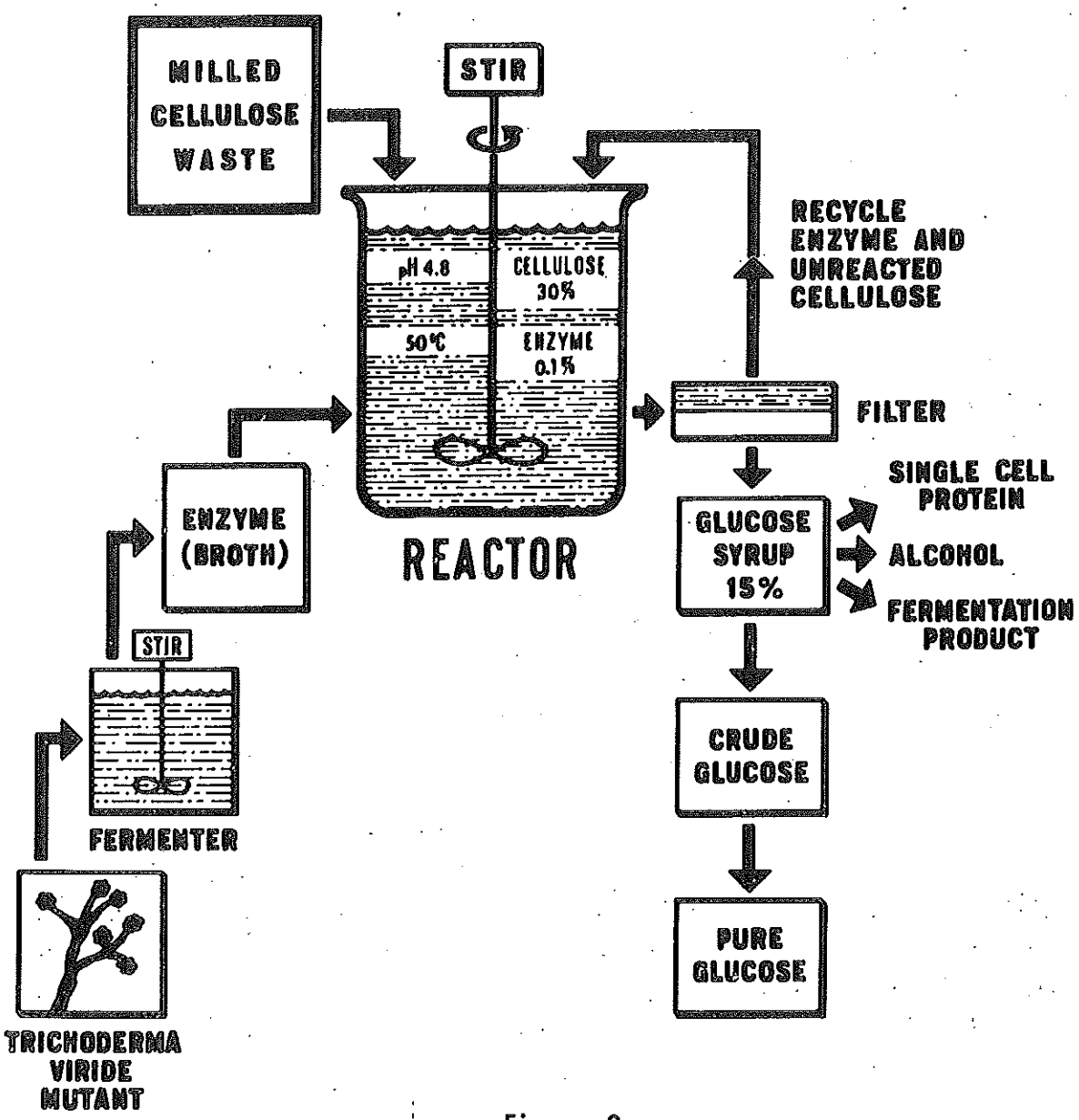

Figure 2

The key to this process is production of a high quality cellulase enzyme complex from *Trichoderma viride* capable of hydrolyzing insoluble crystalline cellulose. This enzyme complex consists of two major components, C_1 and C_X. The C_X component consists of exo and endo β, 1, 4, glucanases. These enzymes are very common and they rapidly attack amorphous cellulose or soluble derivatives such as carboxymethyl cellulose (CMC) producing glucose and cellobiose.

The C_1 is an enzyme required along with C_X for the hydrolysis of insoluble and particularly crystalline cellulose. The action of C_1 is not yet clear although it has been separated from C_X and it is a protein. The simplest explanation and the one held by E. T. Reese (13) is that it is a prehydrolytic enzyme, i.e., it decrystallizes or hydrates cellulose chains so that C_X can act upon them.

Figure 3 graphically shows a crystalline portion of a fiber with close packed, hydrogen-bonded molecules. C_1 has acted on the surface of these to cleave the chain thereby setting free end-portions of the molecules, and permitting them to become fully hydrated. The C_X components are now able to catalyze the hydrolysis of these to glucose.

C_X enzymes are fairly common but C_1 enzymes are quite rare. The best source known is *Trichoderma viride*. When considering large scale hydrolysis of cellulose, C_1 is the limiting factor, consequently, it is essential to use cellulases containing both C_1 and C_X for effective saccharification. Most commercial cellulases are obtained from *Aspergillus niger* and contain chiefly C_X with only traces of C_1. The cellulase produced by *Trichoderma viride* is rich in C_1 and endo β, 1, 4 glucanase. It also contains lower levels of exo β, 1, 4 glucanase and β glucosidase.

During the past twenty years, extensive studies of *Trichoderma viride* and its enzyme have been made at the Natick Development Center in connection with the program on prevention of deterioration of cellulosic materials. Today we are interested in accelerating the breakdown of cellulose. To date, we have defined the conditions needed to produce the enzyme in quantity. We have also developed mutant strains that produce 2 to 4 times as much cellulase as the wild strain. In this area we believe that we have yet to reach the upper limit.

As indicated earlier, the insolubility and crystallinity of pure cellulose and the presence of lignin in waste cellulose make it a most resistant substrate. The most satisfactory

CELLULOSE STRUCTURE SHOWING CRYSTALLINE AND AMORPHOUS COMPONENTS

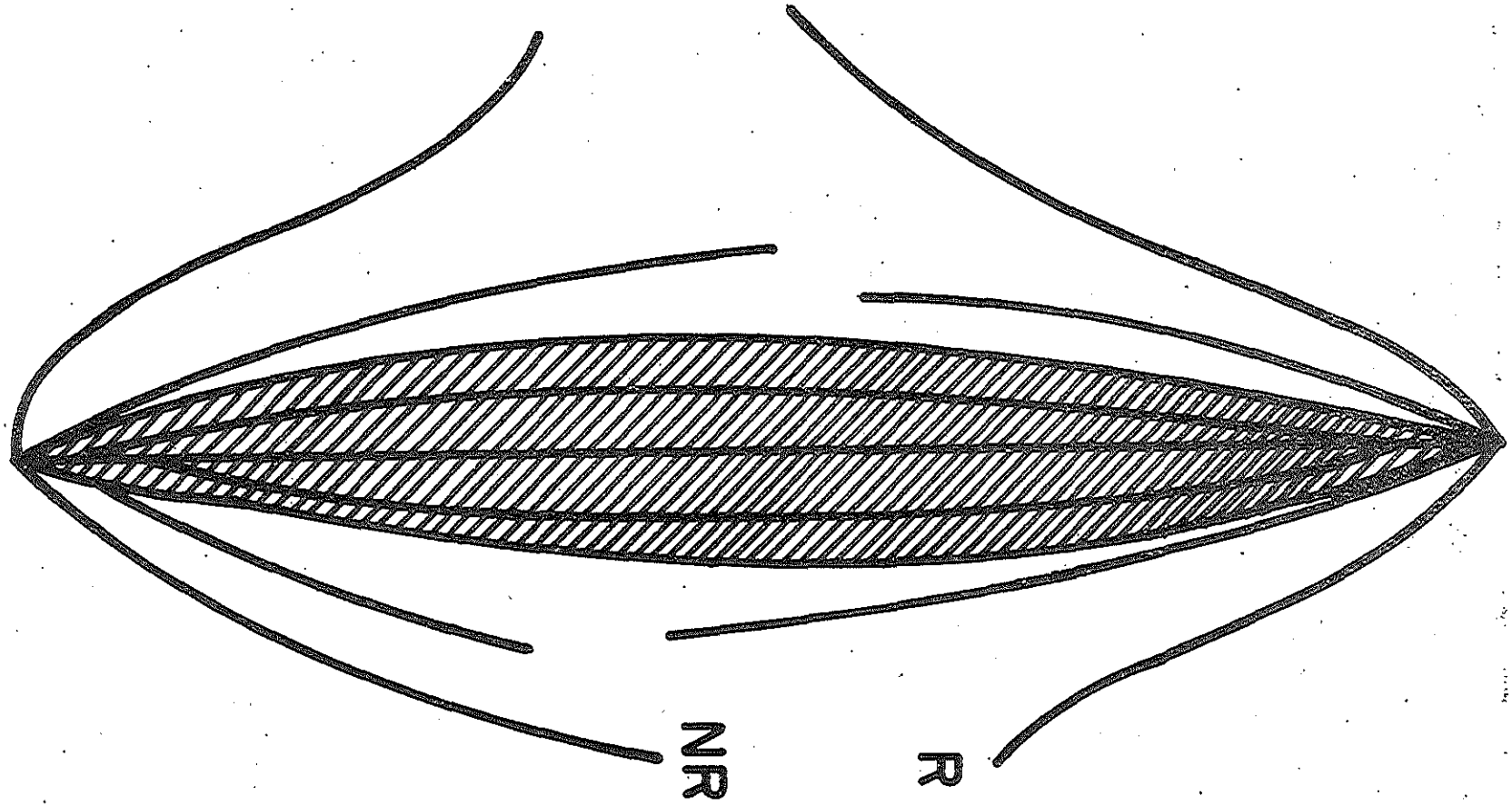

Figure 3

pretreatment we have found is ball milling. This reduces the crystallinity and particle size of the cellulose and increases its bulk density. Consequently, more reactive cellulose is available for saccharification in the reactor.

Pestaloptiopsis westerdijkii QM 381 (PW) produces a cellulase containing largely C_X. Consequently, culture filtrates from this organism can hydrolyze only the amorphous or available portions of the cellulose. This is shown in Figure 4. The amount of available cellulose in fibrous cotton is less than 1%. By ball milling, the availability of cellulose is increased to 11%. Pure cellulose pulp, SW 40, has approximately 7% available cellulose. Through ball milling, this value is increased to 12%. The available cellulose of a commercial pulp BW 200 is increased from 12% to 24% by milling it to 270 mesh (SWECO 270). The cellulose availability in hammer milled newspapers NEP 40, is approximately 6%. Ball milling increases this value to 25% (NPM).

When these same substrates are hydrolyzed by the cellulase broth containing C_1 and C_X produced by *Trichoderma viride* QM 9414 (TV), the available or amorphous portion of the cellulose was hydrolyzed very rapidly. Hydrolysis of the crystalline region followed at a less rapid rate. This is shown in Figure 5.

Total hydrolysis in forty-eight hours ranged from about 6% for fibrous cotton to over 90% for milled pulp, SWECO 270. Milled newspaper was 70% hydrolyzed. Since newspaper is 30% lignin, the 70% hydrolysis represents total hydrolysis of the cellulose content of the newspaper. It is thus apparent that the rate and extent of hydrolysis depends both on the quality of the enzyme used, and the nature and the pretreatment of the substrate.

More conclusive evidence as to the important effect C_1 has on the hydrolysis reaction is shown in Figure 6. Using filter paper as the substrate and enzyme solutions with equal C_X activity as adjusted on carboxymethyl cellulose (CMC), the concentration of glucose produced as a function time was determined. The results show that the enzyme solution containing both C_1 and C_X produced 5 to 6 times more glucose than the enzyme solution containing only the C_X component. The C_X (PW) enzyme rapidly hydrolyzes the limited amorphous portion of the substrate but then hydrolysis stops since it cannot attack the crystalline portion of the substrate. The $C_1 + C_X$ (TV) cellulase attacks the amorphous portion rapidly and then continues to hydrolyze the crystalline cellulose but at a slower rate.

HYDROLYSIS OF INSOLUBLE CELLULOSE BY A Cx CELLULASE FROM PESTALOTIOPSIS WESTERDIJKII

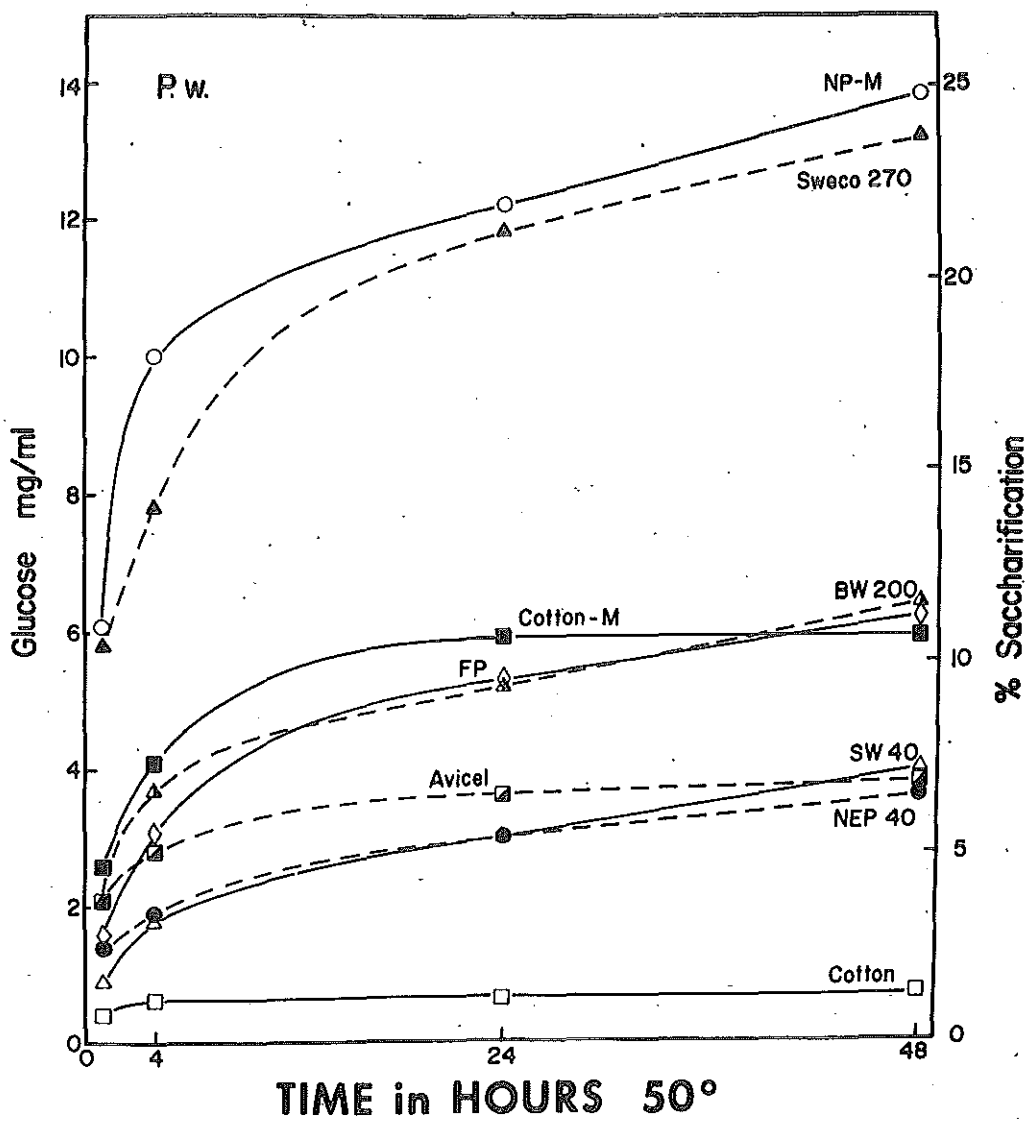

Figure 4

- ○ - NEWSPAPER, GROUND IN A SWECO BALL MILL
- ▲ - PURE CELLULOSE PULP, GROUND TO 270 MESH IN SWECO BALL MILL
- ■ - BALL MILLED ABSORBENT COTTON
- ▲ - BW 200, A BALL MILLED PULP PREPARED FROM SW40, 200 MESH, BROWN CO., BERLIN, N. H.
- ◊ - WHATMAN NO. 1 FILTER PAPER
- ▨ - AVICEL. MICROCRYSTALLINE CELLULOSE, AMERICAN VISCOSE CO
- ● - NEP 40 HAMMER MILLED UNINKED NEWSPRINT 40 MESH
- △ - SW 40. HAMMER MILLED SULFITE PULP, 40 MESH
- □ - ABSORBENT COTTON, FIBROUS

HYDROLYSIS OF INSOLUBLE CELLULOSE BY A COMPLETE CELLULASE FROM TRICHODERMA VIRIDE

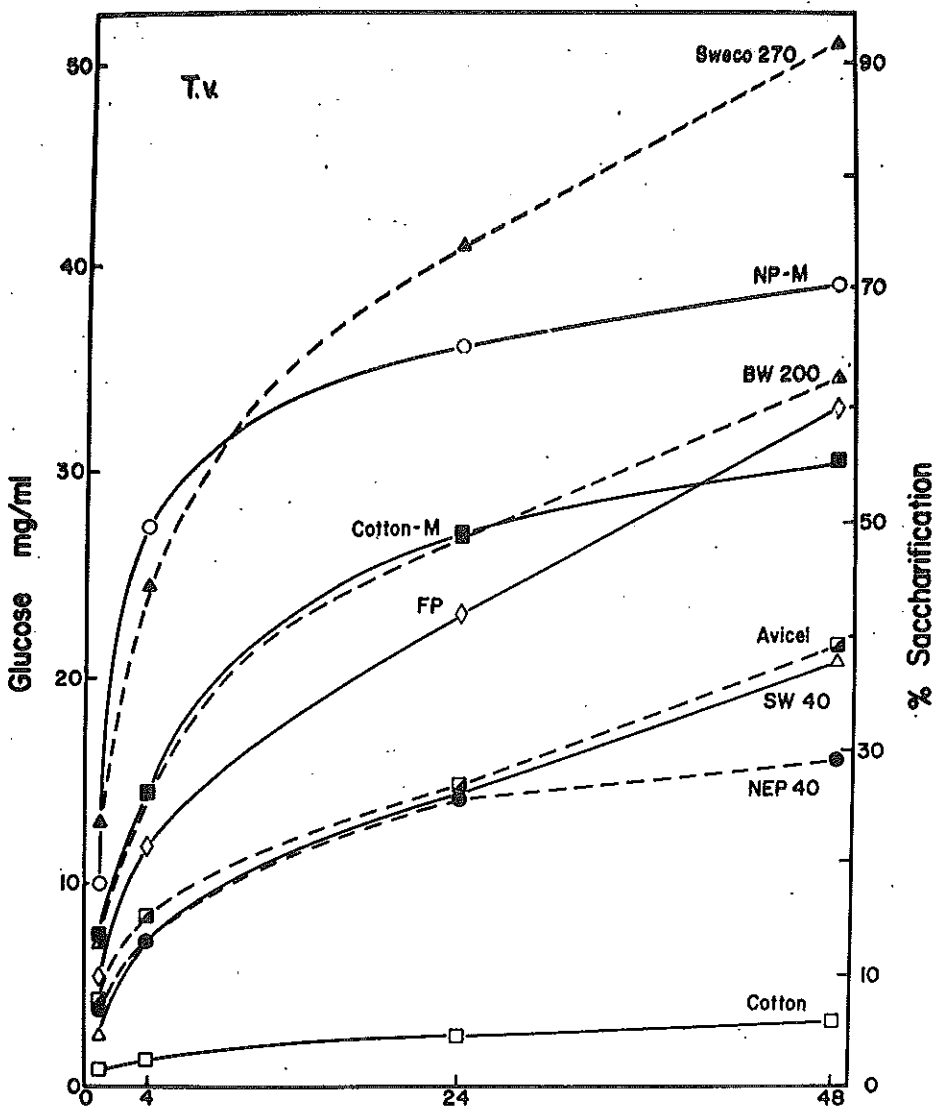

Figure 5

- NEWSPAPER, GROUND IN A SWECO BALL MILL
- PURE CELLULOSE PULP, GROUND TO 270 MESH IN SWECO BALL MILL
- BALLED MILLED ABSORBENT COTTON
- BW 200, A BALL MILLED PULP PREPARED FROM SW40, 200 MESH, BROWN CO., BERLIN, N. H.
- WHATMAN NO. 1 FILTER PAPER
- AVICEL. MICROCRYSTALLINE CELLULOSE, AMERICAN VICOSE CO
- NEP 40 HAMMER MILLED UNINKED NEWSPRINT 40 MESH
- SW 40. HAMMER MILLED SULFITE PULP, 40 MESH
- ABSORBENT COTTON, FIBROUS

HYDROLYSIS OF FILTER PAPER BY CELLULASE PREPARATIONS FROM TRICHODERMA VIRIDE AND PESTALOTIOPSIS WESTERDIJKII ADJUSTED TO EQUAL ACTIVITIES ON CARBOXY METHYL CELLULOSE (19 Cx units ml)

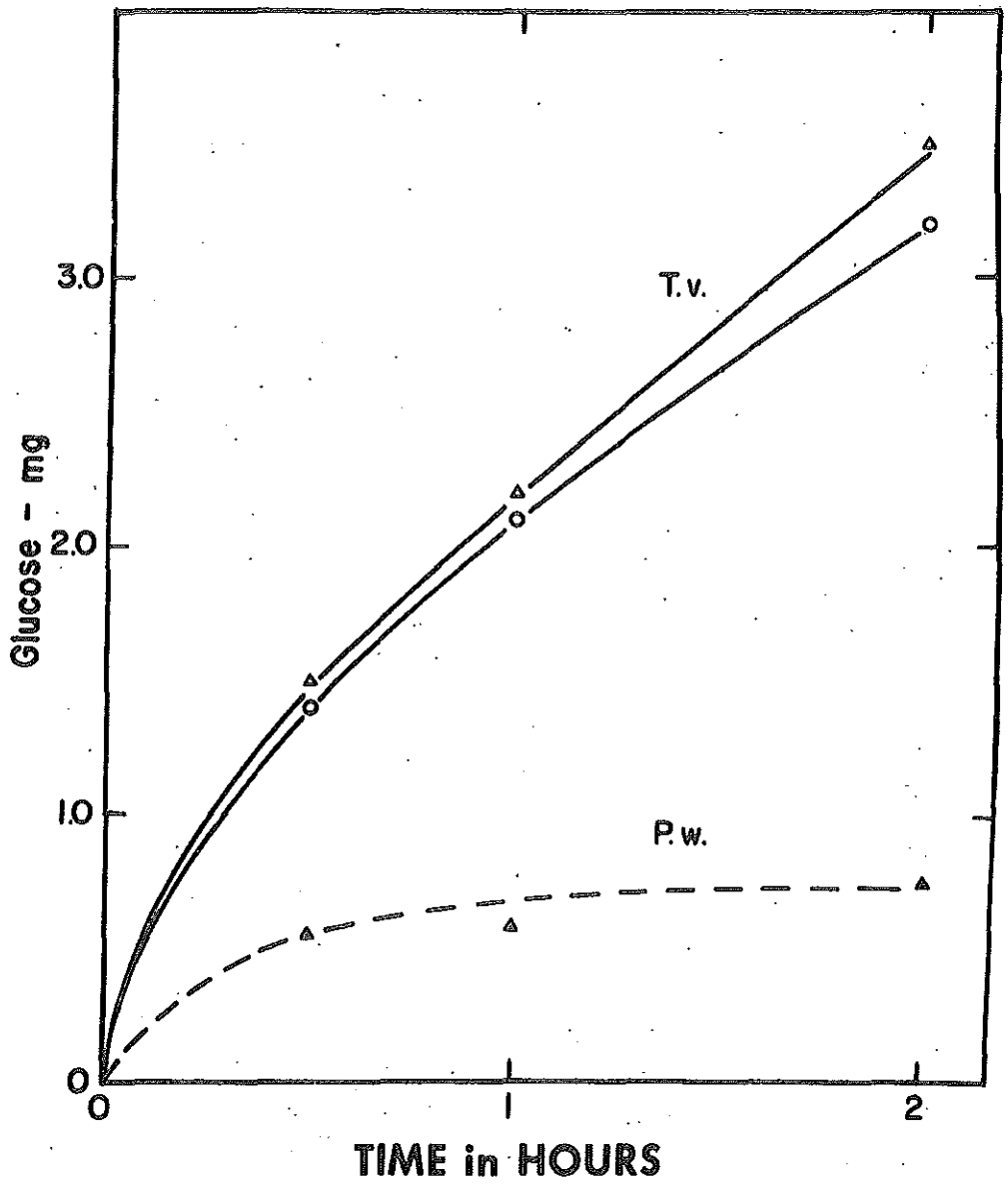

Figure 6

KEY (R-4)
△ - Tv QM 9123 CULTURE FILTRATE
○ - Tv QM 9414 CULTURE FILTRATE
▲ - Pw QM 381 CULTURE FILTRATE

Figure 7 shows the hydrolysis of a number of pure and waste celluloses by the culture filtrate of *Trichoderma viride*. Saccharification is slow for crystalline cellulose such as cotton or untreated rice hulls or bagasse. Pot milling greatly increases their reactivity. Shredded or milled papers make good substrates. The Black-Clawson fiber fraction separated from the hydropulping operation of municipal trash, is an excellent material especially after milling. The same is true for the shredded cellulose fraction separated by air classification of municipal trash by the bureau of Mines' resources recovery process. Waste cellulose from municipal trash is of particular interest because such waste will be increasingly available in large quantity and on a continuous basis.

Pretreatment of the substrate is an important variable which will affect not only the degree of saccharification, but also the economics of the process. Using newspaper as a model substrate, various techniques were tried and the results are shown in Figure 8. It should be noted from these studies that pot milling and ball milling proved best.

Because of its specificity, the cellulase enzyme reacts solely with the cellulose and does not attack other impurities present in the waste. Figure 9 shows the results achieved with milled newspaper digested in a stirred tank reactor. Glucose syrups of 2 to 10% concentrations were realized. The ink, lignin, and other impurities present did not cause any problems (15). The residue after hydrolysis was a black sticky material that dried to a hard nonwettable cake. This material is chiefly lignin which can be burned as a fuel or used as a source of chemicals.

Results achieved with newspaper show that it is technically feasible to produce glucose syrups in good yield and at a fair rate from waste cellulose. Newspapers were selected as the model substrate since such waste is respresentative of cellulosic waste present in municipal trash.

Besides those wastes shown in Figure 7, several other industrial and agricultural wastes have been evaluated and classified as potential substrates for hydrolysis (18). Using ball milled newspaper as representative of all wastes that could be used in the process, the degree of saccharification of other wastes tested relative to that achieved with ball milled newspapers are listed in Figures 10 and 11. Substrates whose relative value is 1.0 or better are considered good substrates for hydrolysis.

HYDROLYSIS OF CELLULOSE BY TRICHODERMA VIRIDE CELLULASE

Substrate	% SACCHARIFICATION 1 hr	4 hr	24 hr	48 hr
PURE CELLULOSE				
Cotton — Fibrous	1	2	6	10
Cotton — Pot Milled	14	26	49	55
Cellulose Pulp SW40	5	13	26	37
Milled Pulp Sweco 270	23	44	74	92
WASTE CELLULOSE				
Bagasse	1	3	6	6
Bagasse - Pot Milled	14	29	42	48
Corrugated Fibreboard Mighty Mac	11	27	43	55
Corrugated Fibreboard Pot Milled	17	38	66	78
Black Clawson Fibers	5	11	32	36
Black Clawson Pot Milled	13	28	53	56
Bureau of Mines Cellulose	7	16	25	30
Bureau Mines Pot Milled	13	31	43	57

Figure 7

PRETREATMENT OF NEWSPAPER

	% Saccharification			
	1 hr	4 hr	24 hr	48 hr
Boiled in Water	4	9	21	26
Cuprammonium	18	35	52	58
Fitzpatrick (Hammer Mill)	10	16	25	28
Gaulin (Colloid Mill)	9	17	27	31
Granulator-Comminutor	6	14	24	26
Jay Bee-Paper Shredder	6	12	24	27
Majac (Jet Pulverizer)	11	15	26	29
Mighty Mac-Mulcher	10	24	31	42
Pot Mill	18	49	65	70
Soaked in Water	7	13	24	28
Sweco Mill	16	32	48	56
Treated 2% NaOH	8	14	28	35
Viscose	15	30	44	51

Figure 8

HYDROLYSIS OF MILLED NEWSPAPER IN STIRRED REACTORS

Enzyme Protein mg/ml	Newspaper %	Temp C	Glucose 1 hr %	4 hr %	24 hr %	48 hr %	Saccharification %
0.7	5	50	1.0	2.0	2.8	-	50
0.7	5	50	1.0	2.0	2.3	-	42
1.0	10	50	2.1	3.1	5.5	7.3	66
1.6	10	45	2.0	3.6	5.4	6.5	59
1.6	10	50	2.3	4.2	6.4	6.3	57
0.8	15	45	1.5	2.8	5.3	7.7	46
0.8	15	50	0.8	2.8	6.1	6.3	38
1.8	15	50	3.2	6.0	8.6	10.0	60

Reactor Volume 1 Liter Stirred 60 RPM pH 4.8

Figure 9

WASTES FOR CONVERSION

SUBSTRATE 5% DRY WT	*RELATIVE % SACCHARIFICATION 24 HOURS		
	AS REC'D WET	AS REC'D DRY	BALL MILLED
PURE			
COTTON	—	0.1	0.9
FILTER PAPER	—	0.8	—
CELLULOSE PULP	—	0.5	1.4
AGRICULTURAL			
RICE HULLS	—	0.03	0.4
BAGASSE (SUGAR CANE)	—	0.09	0.9
RUMEN FIBERS (MANURE)	0.2	0.3	1.0
PAPER			
CORRUGATED FIBREBOARD	—	0.9	1.1
COMPUTER PRINT OUT	—	0.9	1.4
KEY PUNCH HOLES	—	0.8	—
MILK CARTON (POLYETHYLENE COAT)	—	1.0	1.1
NEWSPAPER	—	0.6	1.0
MUNICIPAL TRASH FRACTIONS			
BLACK CLAWSON	0.7	0.7	1.2
BUREAU MINES	—	0.6	0.9

*Relative to Ball Milled Newspaper (56% Sacch.)=1.0 (Substrates whose relative value is 1.0 or better are considered good substrates for hydrolysis)

Saccharified at 50° pH4.8 with T. viride QM9414 Cellulase 1.2 units/ml

Figure 10

INDUSTRIAL WASTES FOR CONVERSION

SUBSTRATE 5% DRY WT	% SACCHARIFICATION 24 HOURS RELATIVE *		
	AS REC'D WET	AS REC'D DRY	BALL MILLED
GOOD AS RECEIVED			
22 NICOLET SULFITE PULP	1.2	0.8	1.7
15 HYDROPULPED GOVT. DOCUMENTS	1.3	1.3	1.5
16 HYDROPULPED GOVT. DOCUMENTS	1.1	0.9	1.5
21 NICOLET KRAFT PULP	—	0.8	1.5
12 KIMBERLY CLARK TISSUE MILL WASTE	1.0	1.0	1.1
1 ST. REGIS PAPER MILL SLUDGE	1.0	0.9	0.9
2 ST. REGIS GLASSINE (PVD) WASTE	—	0.8	0.9
3 ST. REGIS GLASSINE (WAX) WASTE	—	0.8	0.6
GOOD IF BALL MILLED			
13 COTTON LINTERS (MILES)	—	0.2	1.3
18 COREY PAPER MILL WASTE	0.5	0.3	1.2
14 EXTRACTED OAT HULLS (HOFFMAN LA ROCHE)	—	0.1	1.2
20 NICOLET WASTE FILLER	0.6	0.5	1.1
23 RYE GRASS STRAW (MILES)	—	0.3	1.1
17 COVEY PAPER MILL WASTE	0.6	0.5	1.0
26 HERCULES WOOD CHIPS	—	0.1	1.0
25 WELCHES SEEDLESS GRAPE POMACE	—	0.6	0.9
19 STULEY CORN FIBER	—	0.3	0.8
24 WELCHES GRAPE POMACE	—	0.5	0.7

* Ball Milled Newspaper (Ave 42% Sacch) = 1.0

Saccharified at 50° ph 4.8 with T. Viride QM9414
Cellulase 0.08-1.5 w/ml (Ave 1.0)

Figure 11

Milling of the substrate to reduce its crystallinity is an energy intensive and costly process. Consequently, an intensive search for other physical, chemical or combinations of both treatments must be explored to optimize the economics of the overall process.

A potential approach to reducing the cost of substrate pretreatment may be the substitution of hydropulping for the ball milling operation. Preliminary studies with hydropulped government documents show that hydropulping may prove to be very effective as a substrate pretreatment. Saccharification studies using hydropulped substrates at three cellulose slurry concentrations and at three enzyme activity levels were conducted recently and the results are shown in Figure 12. The weight of glucose and percent saccharification realized as a function of reaction time at three enzyme activity levels are shown in Figure 13, 14, and 15. Figure 16 shows the weight of glucose produced and percent saccharification realized as a function of enzyme activity level for a fixed reaction time, i.e., twenty-four hours.

Final glucose concentrations ranged between 1.6 to 4.6% and increased with either enzyme activity or substrate concentration. Final percent saccharification ranged from 33 to 77% and increased with enzyme activity, but decreased as the substrate concentration increased.

At Natick we have been preferring milling as a means of increasing the reactivity of the raw materials; others prefer chemical pretreatment. Toyama, for example, obtains results comparable to ours by using an alkaline pretreatment of bagasse. The costs of milling and of chemical pretreatment appear to be of the same order of magnitude.

These encouraging results obtained with hydropulped substrates may prove to be most significant in cutting substrate pretreatment costs and thereby improving the overall economics of the process.

Having proved that this process is technically feasible, our next step is an intensive pre-pilot plant study to optimize all variables and to obtain the engineering and economic data needed for the design of a demonstration plant.

In collaboration with Fermentation Design, Inc. of Bethlehem, Pa., we have engineered a highly instrumented pre-pilot plant consisting of such equipment as:

HYDROLYSIS OF HYDROPULPED GOVERNMENT DOCUMENTS IN 1 LITER STR 50 C PH4.8

SAMPLE NO	ENZ CONC u/ml	S CONC % DRY WT	HYDROLYSIS AT 24 HRS	
			GLUCOSE mg/ml	SACCHARIFICATION %
15	0.5	2.3	16	64
16	0.5	4.8	23	43
16	0.5	7.7	26	30
16	1.0	2.3	20	77
16	1.0	5.0	33	59
16	1.0	7.5	39	47
16	1.5	2.4	21	79
16	1.5	5.2	39	67
16	1.5	7.8	46	53

Enz= Cellulase of T. viride QM9414

S=Pulped Documents As Rec'd - wet

$$\% \text{ Saccharification} = \frac{\text{glucose mg/ml} \times 0.9}{\text{5 mg/ml (original)}} \times 100$$

Figure 12

HYDROLYSIS OF HYDROPULPED GOV'T DOCUMENTS BY TRICHODERMA VIRIDE CELLULASE

[E] = 0.5 units ml (FP)

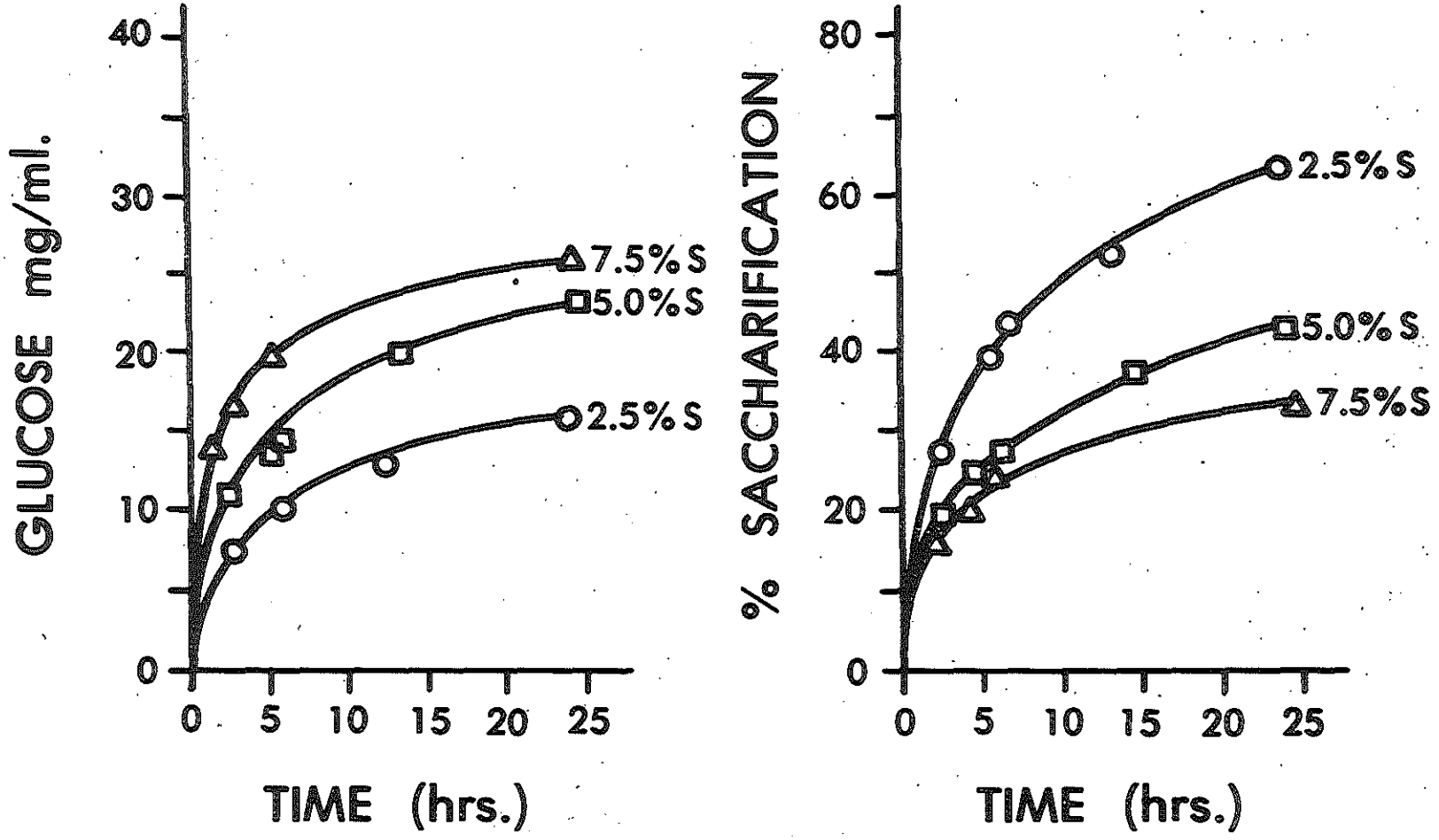

Figure 13

HYDROLYSIS OF HYDROPULPED GOV'T DOCUMENTS BY TRICHODERMA VIRIDE CELLULASE

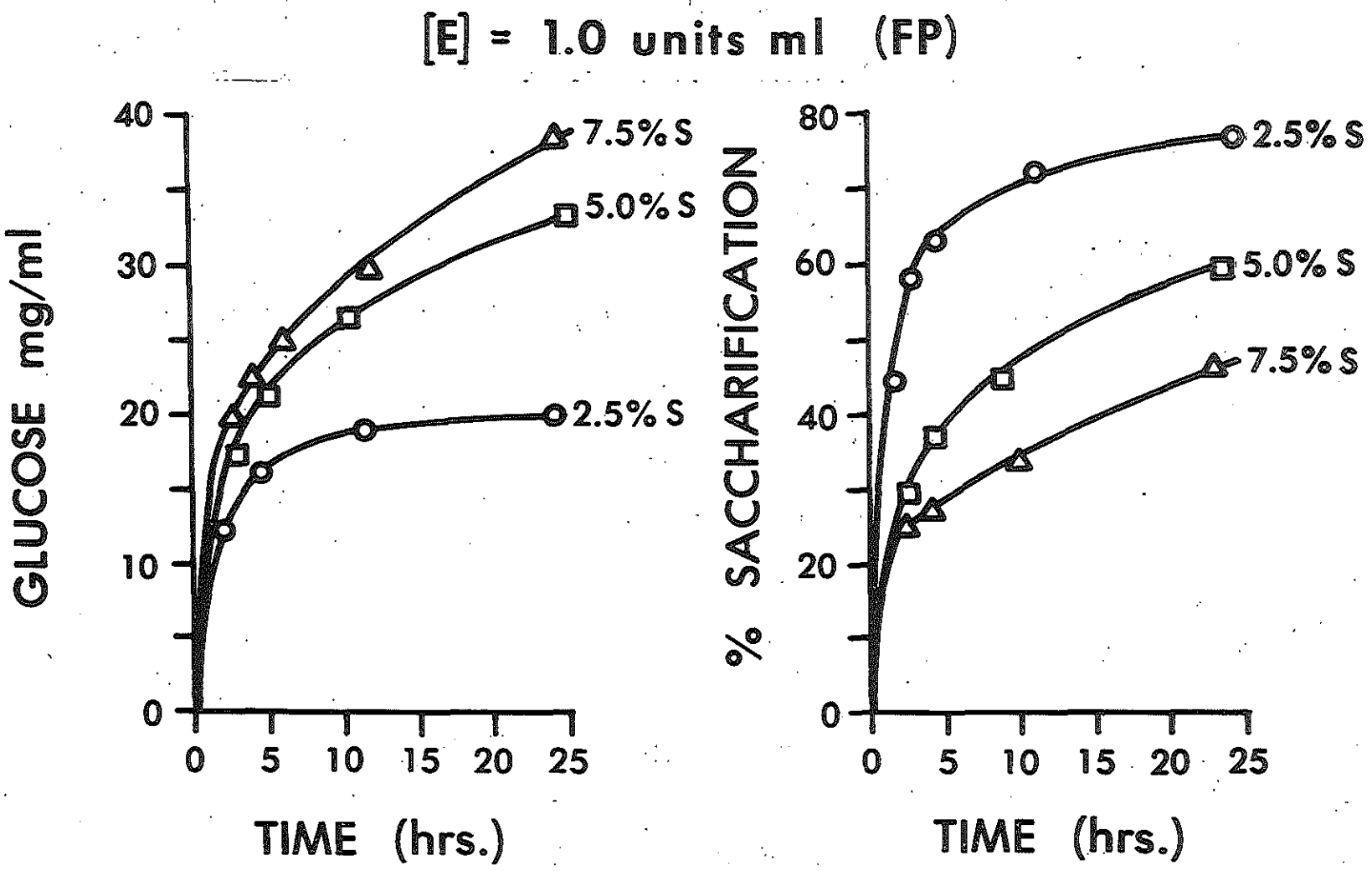

Figure 14

HYDROLYSIS OF HYDROPULPED GOV'T DOCUMENTS BY TRICHODERMA VIRIDE CELLULASE

[E] = 1.5 units ml (FP)

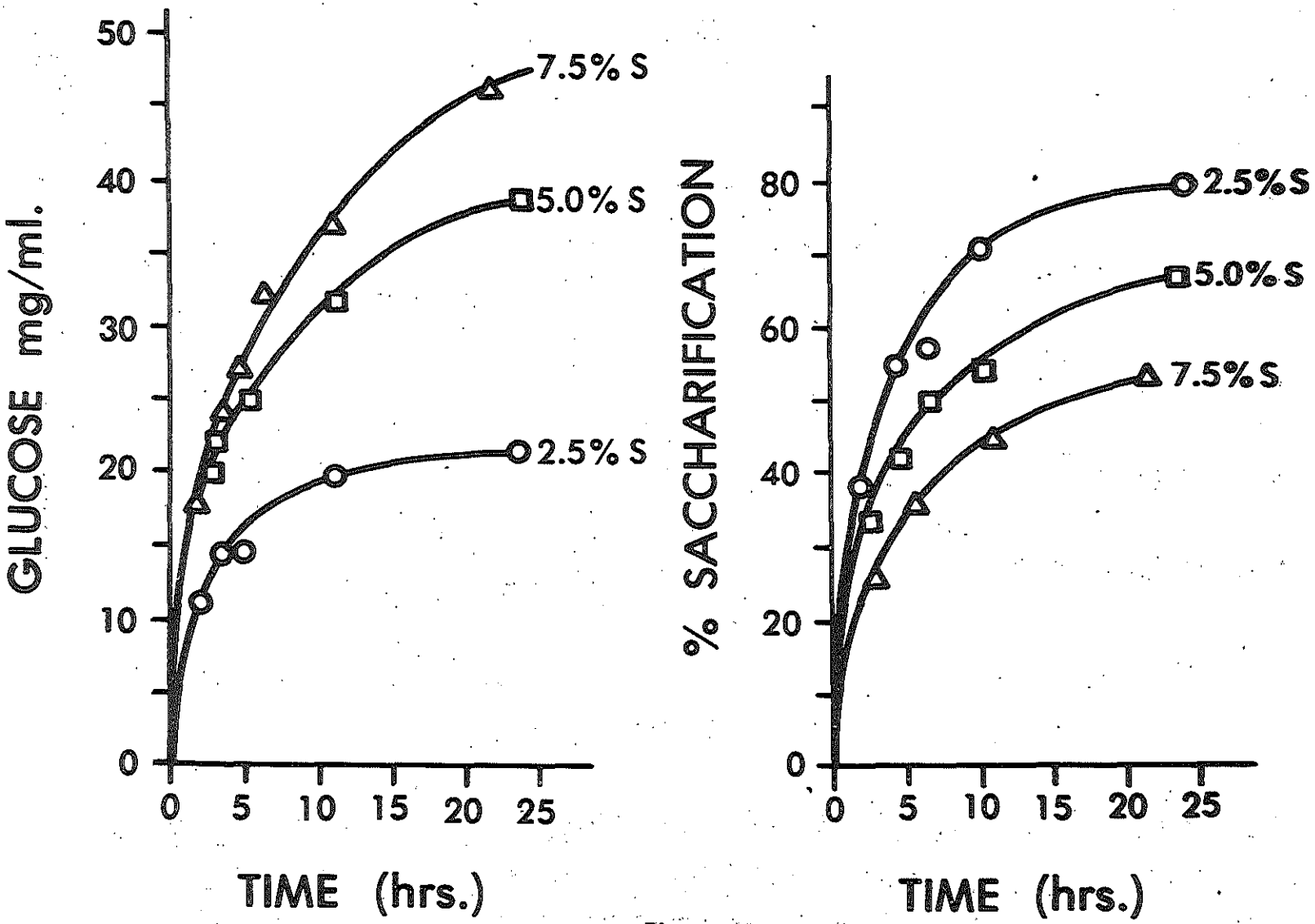

Figure 15

RELATIONSHIP OF T. VIRIDE CELLULASE ACTIVITY LEVEL TO 24 HOUR GLUCOSE YIELD AND PERCENT SACCHARIFICATION IN THE HYDROLYSIS OF HYDROPULPED GOV'T DOCUMENTS

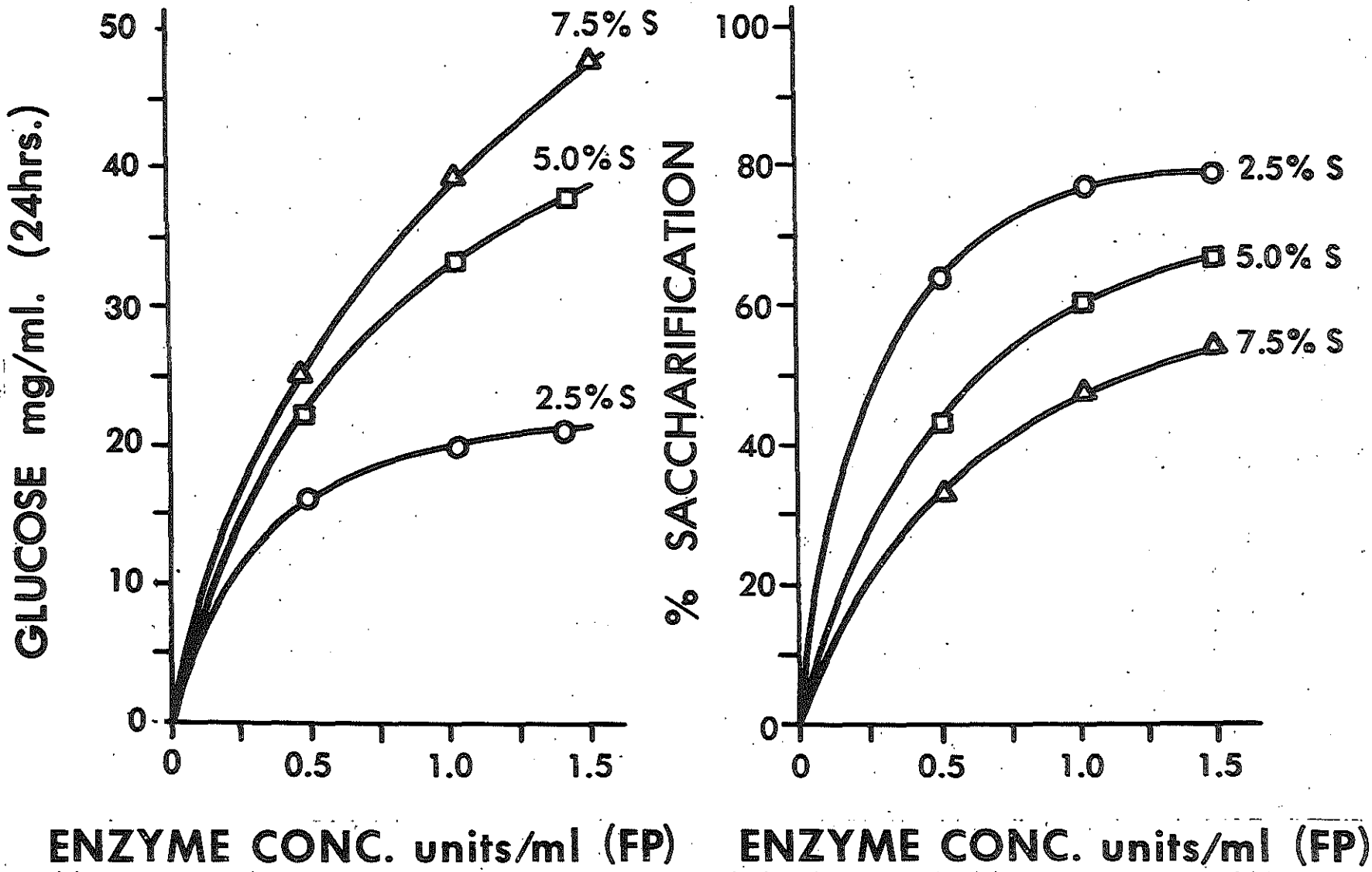

Figure 16

1. Fermenters
2. Enzyme reactors
3. Holding tanks and auxiliary vessels
4. Instrumentation modules
5. Substrate handling and preparation equipment
6. Enzyme recovery and concentration equipment

The design and construction is such that the most sophisticated fermentation techniques including batch, continuous and semi-continuous processes can be studied.

Because of the sophistication of the monitoring and control instrumentation, both the fermentation and the enzyme hydrolysis will be continuously monitored and controlled in order to optimize the output of the individual processes. Figure 17 shows the 250 liter biological reactor that will be used to study the cellulose hydrolysis. Figures 18 and 19 show the 400 liter fermenter with its 30 liter seed fermenter that will be used to produce the cellulase enzyme from the *Trichoderma viride* fermentation. Figure 20 shows the instrumentation cabinets for the fermenter and enzyme reactor which contain modules for control or analysis of temperature, pressure, agitation speed, pH, sparging dissolved oxygen, vessel weight, liquid level, and exit gas.

Figure 21 shows the simplified schematic of the process. The initial capacity of this equipment is the processing of 1000 lbs. of cellulose per month. With minor modifications it may be possible to increase its capacity to two, three, and possibly 4000 lbs/month. This equipment is now operational at Natick.

Upon completion of these studies, it will be possible to engineer with certainty larger pilot demonstration plants and possibly full scale plants.

The potential world-wide impact of this process on the food, energy and ecology problems has been recognized both nationally and internationally.

Requests for information, process data or for the opportunity to visit and observe the process have come from Japan, Indonesia, India, Australia, Italy, Formosa, Venezuela, Guatemala, the Philippines, Mexico, Brazil, England, Finland, New Zealand, France, Sweden, Germany, Hong Kong, Israel, Russia, Canada, Hawaii, Puerto Rico and Malaysia.

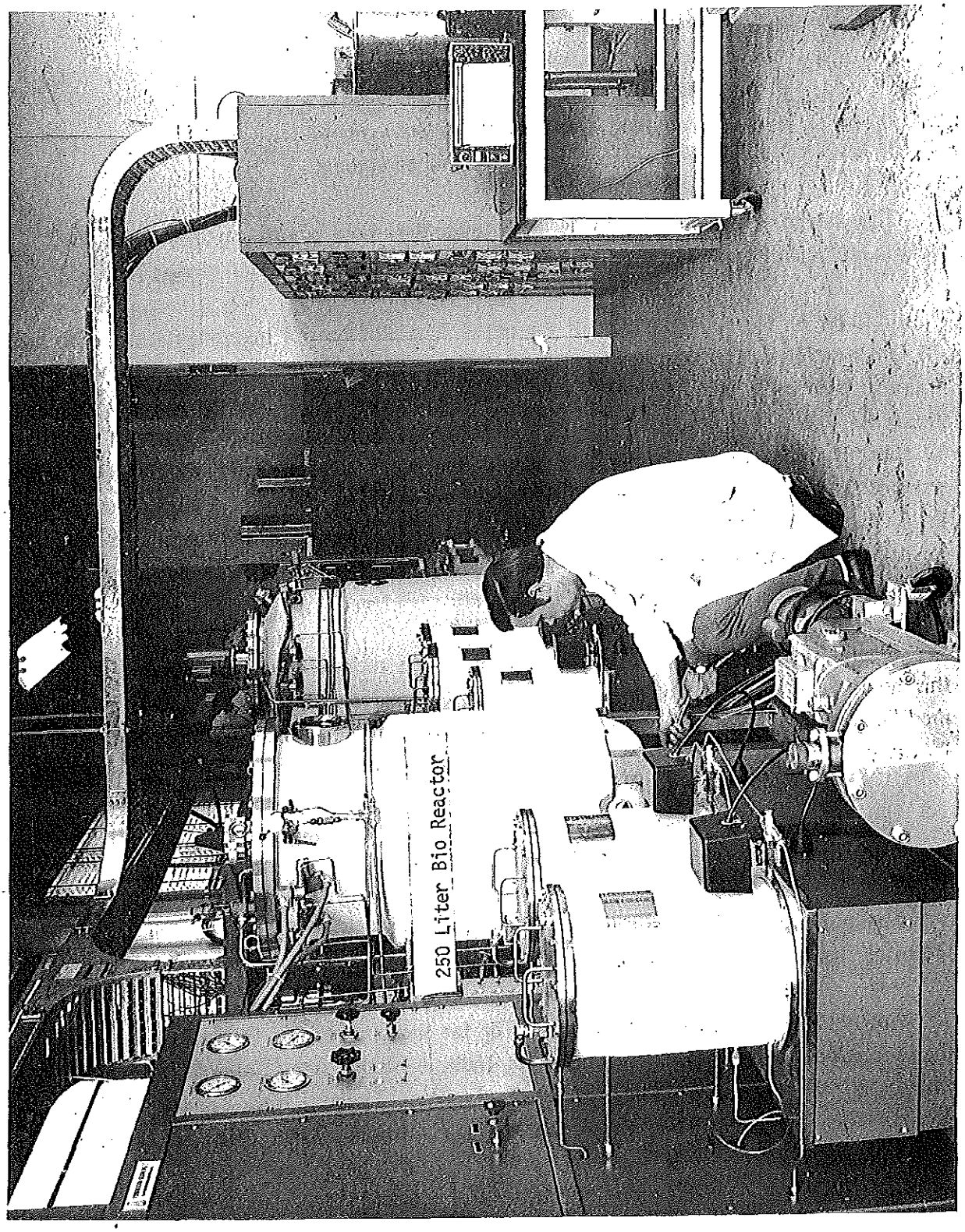

Figure 17

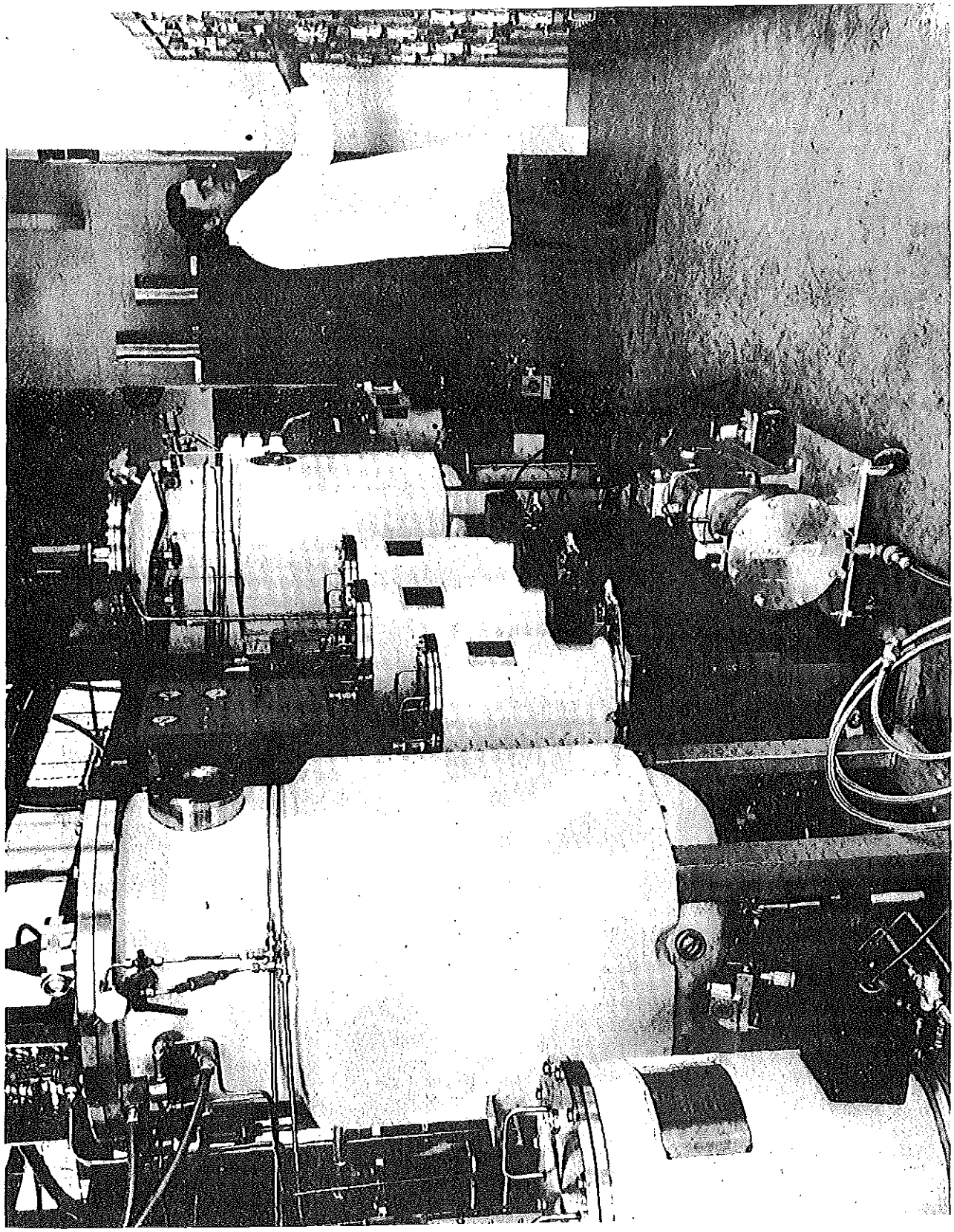

Figure 18

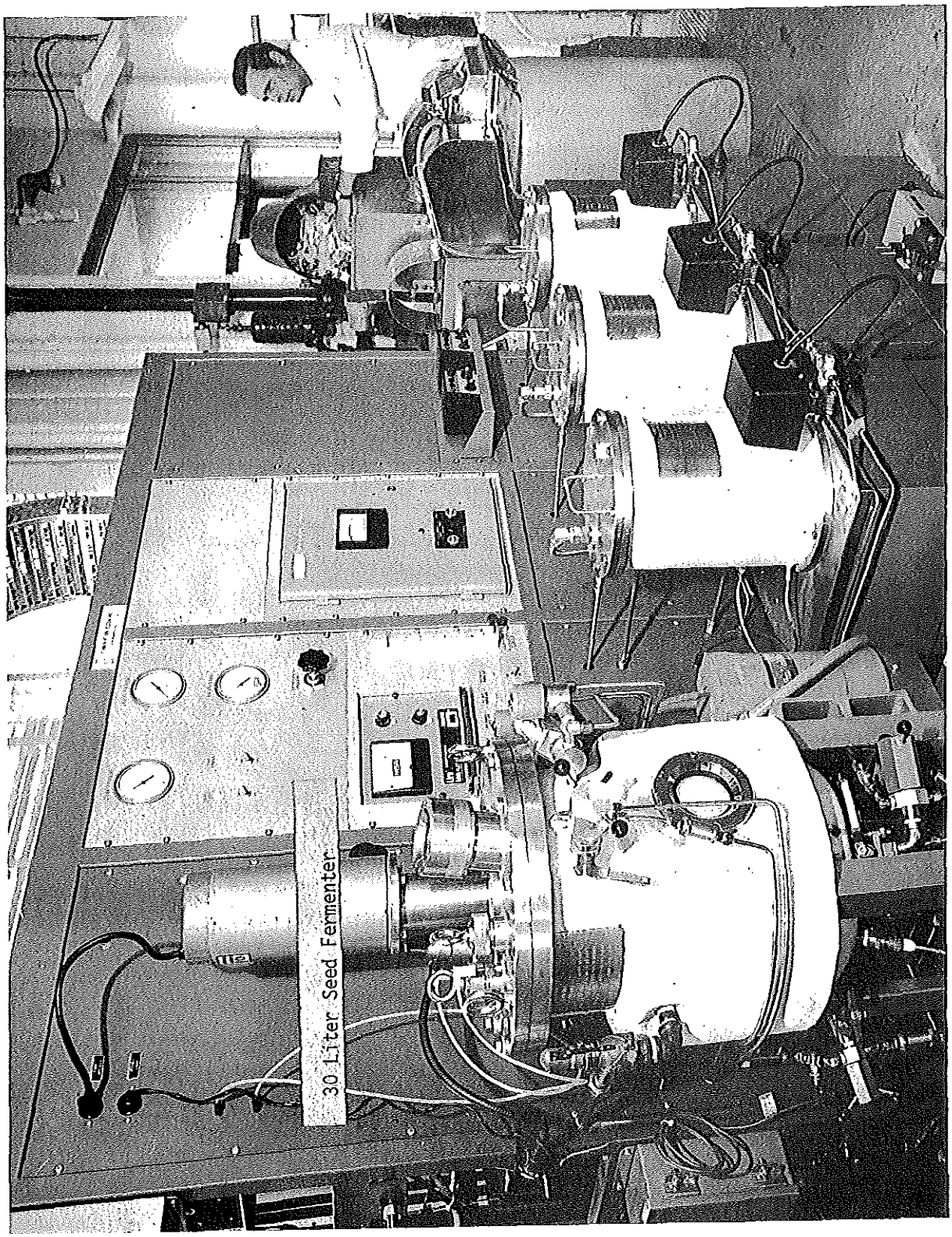

Figure 19

Figure 20

ENZYMATIC CONVERSION OF WASTE CELLULOSE

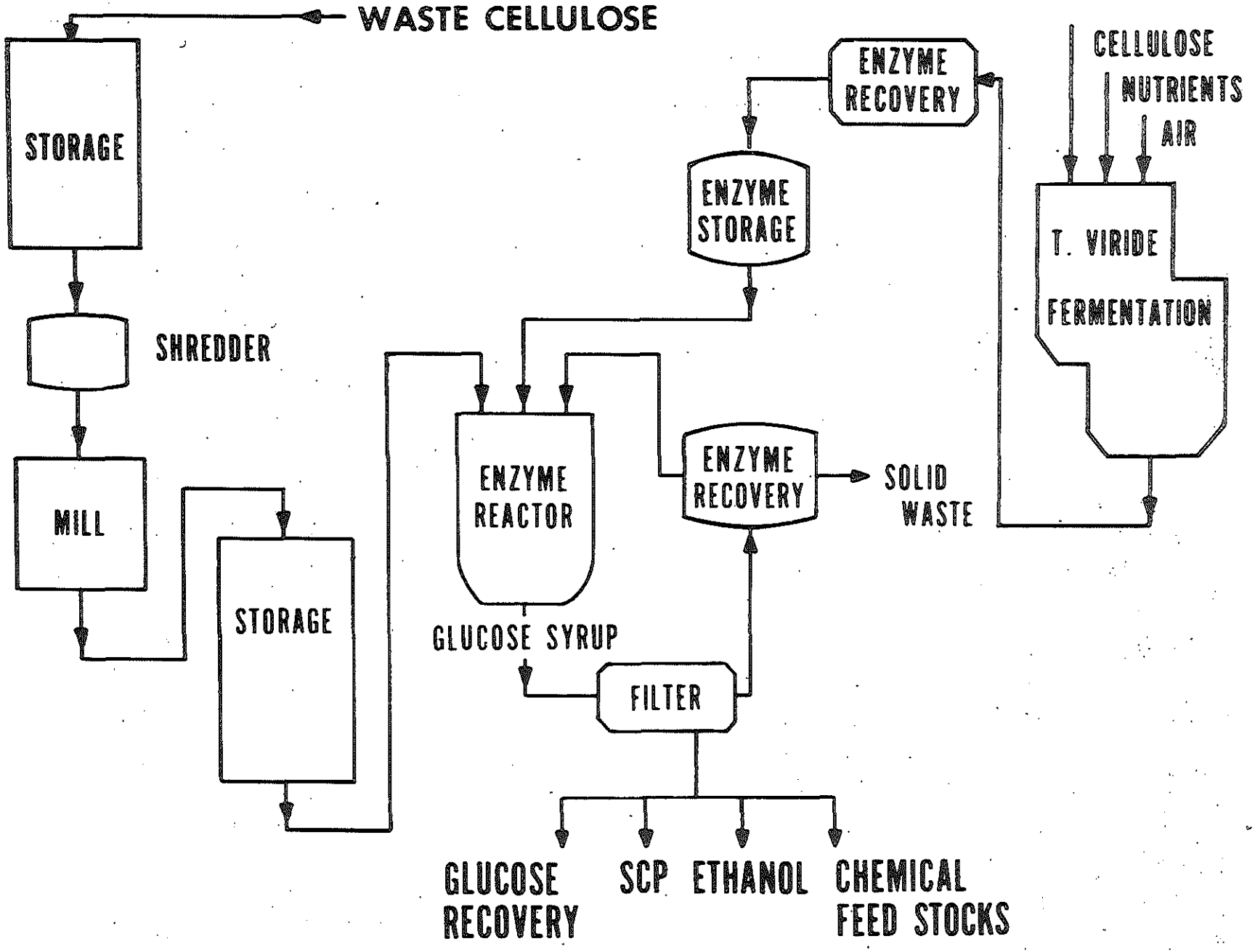

Figure 21

Many U. S. chemical companies, pulp and paper mills, processors of agriculture products and various state and municipal governments have shown definitive interest in the exploitation of this process. Because of this interest, the U. S. Army Development Center are working very closely with several industrial firms to assure the transfer and translation of this new technology to commercial scale as soon as practicable for the benefit of the nation and mankind.

In conclusion we at Natick are convinced that:

1. The vast quantity of cellulose produced annually should be exploited as a source of energy, food, and chemical feedstock.
2. The enzymatic hydrolysis of such energy rich material as cellulose to glucose is technically feasible and practically achievable on a very large scale by 1980.
3. The exploitation of our fossil fuel reserves be it coal, oil shale or other, may satisfy our energy demands for the next five to ten decades, however, we believe that the ultimate long-range solution to the world's energy problem is the development of practical and economical processes capable of harnessing the inexhaustible energy of the sun.

We at Natick Development Center look forward with great expectation and confidence to the opportunity of contributing to the effort that will assist this country and the world in the solution of our pressing food, fuel and ecology problems.

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